tissues, which are assumed to secret some factors under the regulation of nerves causing changes in the tissue stiffness. The tissues contain a large amount of the extracellular matrix mainly consisting of collagen fibrils, proteoglycans and microfibrils. The unique properties of these collagenous tissues might be due to lack of permanent associations between the collagen fibrils and the surrounding extracellular matrix. It seems that cross-linking between the fibrils are formed or broken during the change of the stiffness of the tissues. Its molecular mechanisms are, however, not yet fully understood. We isolated a protein factor called 'tensilin' from an extract of sea cucumber body wall dermis, one of the known catch connective tissues. It stiffens the detergent-treated dermal pieces and induces aggregation of collagen fibrils isolated from the tissue. We also isolated another protein factor which stiffens the dermal pieces. It is possible that there are other factors affecting on interactions among dermal fibrils and the stiffness of the tissues. Molecular mechanisms of the stiffness changes of the catch connective tissues should be clarified by purifying and characterizing these factors.

3711-Pos Board B439

Diffusion Discrepancy between Stroma of Tumor and Normal Tissues

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It is known that stromal microenvironments change in terms of its structure and composition during tumor progresses. Such change can lead to changes of diffusion efficiency and/or orientation of small molecules. Given small molecules such as cytokines and microRNAs are actively involved in tumorigensis, study diffusion in tumor stroma can lead to identifying the mechanism contributing to tumor progression. We used fluorescence recovery after photobleaching (FRAP) to examine the immobile fraction, diffusion rates, diffusion directionalities of dextran between 10 kD and 100 kD of molecular weight in stroma from both normal and tumor tissues from human breasts. We found that in the area with dense fibers, the diffusion rate in the tumor tissue is at least 2 fold-higher compared to the normal tissue. Furthermore, it was observed that 20% more dextran is immobilized in the tumor tissue, compared to the normal tissue, during the time frame of FRAP experiment, indicating the existence of efficient physical traps of small molecules in tumor stroma.

3712-Pos Board B440

Effect of Oligosaccharide Modified Material X on Viability of Human Cancer Cell Lines

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Anticarcinoma agents take part in the selective destruction of cancer cell lines, or inhibit the growth and proliferation of cancer cells. Finding anticarcinoma agents that have do not have noteworthy negative side effects is important matter for application in various flelds. Most of polysaccharides were used as medical product or an additive to health functional food. For example chitin and chitosan are known to exhibit antitumor, antibacterial, and antihypertensive activity. In this study, we incubated two kinds of cancer cells (Hep3B, A549) and 293T HEK cell treated with material X in concentration of 0.5%, 1% and 2% respectively for 24h. Then we measured viability of the cells by MTT assay. Our data suggest viability of 293T decreases gradually as concentration of X increases. Survival rates of 293T with X in concentration of 0.5%, 1% and 2% are 73.3%, 53.2% and 31.3% respectively. The cancer cell lines had more tolerance for 0.5% X and 1% X. However the cancer cells exhibited a rapid decline of viability when treated with 2% X. Survival rate of Hep3B with 2% X is 8.7% and that of A549 is 8.4%. With 2% X, cancer cell lines are about 4 times cytotoxic effects of the normal cell line. These results indicate that material X of specific concentration or higher depletes cancer cell lines while showing gradual effect on normal cell lines. To our knowledge, material X could be a promising antitumor application.

3713-Pos Board B441

Cytotoxic Effects of Substance a Obtained from Oligosaccharides on Human Lung Cancer Cell Line, A549

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It is reported that oligosaccharides have antitumoric effects on cancer cells. To evaluate the in vivo antitumor potentials of oligosaccharides, we obtained substance A from them. In this study, we treated three kinds of cell lines(Hep3B, A549, 293T) with substance A in concentration of 2%, 1%, 0.5% respectively and incubated them 24 hours. Then we used MTT assay to measure viability of cells. As a result, the viability of A549 cells decreased as the concentration of substance A became higher while other cells(Hep3B, 293T) were almost same. Survival rates of A549 cells were 84%, 82% and 68% when treated with substance A in concentration of 0.5%, 1% and 2% respectively. In all concentrations, cytotoxic effects of substance A on A549 cells were about 5 times stronger than on 293T cells. Our results suggested that substance A has selective cytotoxic effects on A549 cell lines. This study demonstrated that substance A has antitumor effects on human lung cancer.

3714-Pos Board B442

In Vivo Studies of Active Processes in the Escherichia Coli Nucleoid

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The cell is the site of actively motor-driven processes which drive the intracellular environment far from thermodynamic equilibrium. The dynamics of biological macromolecules such as DNA in such an environment are complex and subject to a multitude of constraints and forces. Inspired by our in vitro studies of DNA looping with optical tweezers that showed that additional non-thermal fluctuations in the DNA can substantially enhance the formation of regulatory DNA-protein complexes, we study the conformational fluctuations of chromosomal DNA in vivo in Escherichia coli by Fluorescence Correlation Spectroscopy (FCS).
Conformational fluctuations of the DNA move the bound fluorophores stochastically into the diffraction-limited excitation volume of a focused laser beam in a confocal microscope. From the time correlation functions of the measured fluorescence intensity, we quantify the fluctuations of the DNA as measured by its time-dependent mean square displacement, and the viscoelastic moduli of the nucleoid. These quantities in live cells significantly differ from the ATP-depleted dead cells on longer time scales, indicating that the fluctuations on longer time scale may be driven by active processes involving molecular motors that generate forces by ATP hydrolysis. On shorter time scales, we see little difference between live and dead cells, suggesting that the processes on corresponding short length scales rely primarily on thermally-driven diffusive mechanisms. We also note that the rheological properties of E. coli nucleoid significantly change when the ATP hydrolysis in cells is inhibited.

3715-Pos Board B443

The Energetic Contribution of Water in the Binding of Ribonuclease a and UMP

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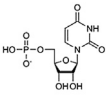
In most treatments of aqueous binding reactions, the energetic contribution of water is not addressed explicitly by the governing equation. The classical equation for a binding equilibrium ($\Delta G^\circ = -RT\ln K$) may be appropriate at infinite dilution but not under experimental conditions, especially in "nonideal" solutions containing other solutes. Resolving this issue is paramount to understanding the thermodynamics of molecular interactions in the context of a living cell. In the current study, we test a new equation that treats water as a co-reactant and co-product of the balanced reaction. The binding affinity of ribonuclease A (RNase A) with an inhibitor molecule, uridine-3'-monophosphate (UMP), is quantified using isothermal titration calorimetry. The results indicate that the equilibrium "constant," K, is dependent on reactant concentration and that the desolvation energy of binding is unfavorable for this specific protein-ligand interaction. These observations are consistent with published findings for another model binding system, the chelation of calcium by EDTA (*J. Phys. Chem. B* 2013, 117, 8180).


(RNase A)


(UMP)

3716-Pos Board B444

Size, Stoichiometry, and Organization of Soluble LC3-Associated Complexes

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Microtubule associated protein 1 light chain 3B (LC3/ATG8) functions in autophagosome formation and autophagy substrate recruitment. LC3 exists in both a soluble (autophagosome-independent) form as well as a lipid modified form that becomes tightly incorporated into autophagosomal membranes. Although LC3 is known to associate with tens of proteins, relatively little is known about soluble LC3 aside from its interactions with the LC3 lipid conjugation machinery. In previous studies we found autophagosome-independent GFP-LC3 diffuses unusually slowly for a protein of its size, suggesting it may be constitutively associated with a high molecular weight complex, form homo-oligomers or aggregates, or reversibly bind microtubules or membranes. To distinguish between these possibilities, we characterized the size, stoichiometry, and organization of autophagosome-independent LC3 in living cells and in cytoplasmic extracts using Fluorescence Recovery After Photobleaching (FRAP) and Fluorescence Polarization Fluctuation Analysis (FPFA). We found that the diffusion of autophagosome-independent LC3 was unaffected by either mutational disruption of its lipid modification or microtubule depolymerization, suggesting this form of LC3 does not reversibly bind to microtubules or membranes. Brightness and homoFRET analysis indicate LC3 does not homo-oligomerize, ruling out this as a possibility for its slow diffusion. In contrast, mutation of specific residues on LC3 required for binding other proteins and mRNA led to changes in the effective hydrodynamic radius of the protein as well as its stoichiometry. This suggests that LC3 associates with a multi-component complex consisting of either proteins or RNA. We conclude that autophagosome-independent LC3 associates with a complex with an effective size of ~500 kDa in the cytoplasm. These findings provide new insights into the nature of autophagosome-independent LC3 and illustrate the power of FRAP and FPFA to provide novel insights into the emergent properties of protein complexes in the autophagy pathway.

3717-Pos Board B445

Homeostasis of the Cellular Actin Cortex

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The cell cortex is a thin network of actin, myosin motors, and associated proteins that underlies the plasma membrane in most eukaryotic cells. It enables cells to resist extracellular stresses, perform mechanical work, and change shape. The actin network undergoes constant reorganisation due to molecular turnover. Hence, cortical structural and mechanical properties depend strongly on the relative turnover rates of its constituents and the actin filament length-distribution, but quantitative data on these dynamics remains elusive. We combined single molecule speckle microscopy and photobleaching experiments with microscopic computer simulations to analyse how molecular binding dynamics of G-actin to filaments sets network turnover and consequently the mechanical properties of the cellular actin cortex in living cells. Using photobleaching experiments, we found that two filament families with very different turnover rates composed the actin cortex: one with fast turnover dynamics and polymerisation resulting from addition of monomers to free barbed-ends and one with slow turnover dynamics with polymerisation resulting from formin-mediated filament growth. We show that filaments in the second subpopulation are on average longer than those in the first and that cofilin-mediated severing of formin-capped filaments contributes to replenishing the filament subpopulation with free barbedends. Additionally, we measured the molecular association rates and the distribution of travel-distances of actin monomers and formin dimers in speckle experiments and showed that this travel-distance distribution is consistent with the actin filament length-distribution found from photobleaching experiments and molecular simulations. Together, our results provide a quantitative characterisation of essential mechanisms underlying actin cortex homeostasis.

3718-Pos Board B446

Structural Transitions of Membrane-Bound Chiral Biopolymers

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In vivo, structural biopolymers such as MreB, FtsZ and eukaryotic homologs such as F-actin can exist in a membrane bound state where they assist in and regulate many important cellular functions including cell division and cell wall growth and maintenance. Here we show that the interplay between the chirality of a filament, its elasticity and membrane interactions can have non-trivial consequences for the conformations that it can adopt, thus directly affecting its functional role. We study a continuum model that describes a filament that is bound to an attractive cylindrical (rigid) membrane which extends our analysis for filaments bound to flat substrates (ref-2012). In the model we explicitly treat the natural preferred twist that is inherent in most biopolymers due to monomer stacking and assume that binding domains along the filament follow this natural twist forming a "sticky" attractive helix. We also introduce two elastic parameters allowing for the mechanical compliance of the filament, where filament bending along the surface of cylinder is modeled as a Worm-Like Chain (WLC) that couples to the curvature of the cylinder, and elastic twist deformation along the axis of the filament. We find, for certain parameter regimes, there exists a transition from an absorbed straight filament to a mechanically stable conformation where a helix is preferred. We also find there are periodic surface bound discommensurate stable solutions for both of the bending and twist degrees of freedom, where filament conformations are described by the well studied Frenkel-Kontorova dynamics for absorbed atomic lattices. Our results provide insight into the relation between a filament's molecular properties and its macroscopic conformation in a functional context.

3719-Pos Board B447

***In Vivo* Orientation of Single Myosins in a Zebrafish Embryo**

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Cardiac and skeletal myosin is highly organized in the muscle lattice where it powers contraction by transducing ATP free energy into the mechanical work of moving actin in a mechanism known as transduction/mechanical coupling. While muscle myosin can move actin *in vitro*, its *in vivo* environment is crowded and constrained by the fiber lattice. *In vivo*, myosin side chains are modified during- and post-translation by mutation, phosphorylation, deamidation, and oxidation under normal, diseased, or aging conditions and all potentially impacting transduction/mechanical coupling. Single myosin detection provides highly prized "bottom-up" quantitative characterization of myosin that tests hypotheses without the ambiguities inherent in ensemble derived observations. The marriage of *in vivo* and single myosin detection to study human cardiac or skeletal muscle contraction in zebrafish embryo models is a multi-scaled technology for basic and translational research. It allows one-to-one registration of a selected myosin molecular alteration with muscle filament-sarcomere-cell-fiber-tissue-organ- and organism levels of phenotype with confidence that all interactions and modifications are appropriately contributing their impact to myosin conformation. *In vivo* single myosin lever-arm orientation was observed at super-resolution using a photoactivatable GFP (PAGFP) tagged myosin light chain expressed in zebrafish skeletal muscle. Imaging was aided by an innovative microfluidic design for embryo confinement. Tag specificity was demonstrated by the simultaneous observation of 2-photon fluorescence emission and second harmonic generation (SHG) from myosin. Single molecule detection used highly inclined and laminated optical sheet (HILO) illumination and was verified by quantized photoactivation or photobleaching. Single molecule emission patterns from relaxed muscle indicated a highly orientationally confined lever-arm orientation. Results demonstrate detection of single myosin orientation *in vivo*. The zebrafish muscle system serves as an *in vivo* model for human disease and aging effects on myosin. Research supported by NIH R01AR049277 and R01HL095572.

3720-Pos Board B448

Protein Recognition and Selection through Conformational and Mutually Induced Fit

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Protein-protein interactions drive most every biological process but in many instances the domains mediating recognition are disordered. How specificity in binding is attained in the absence of defined structure contrasts with well-established experimental and theoretical work describing ligand binding to protein. The signaling protein calmodulin presents a unique opportunity to investigate mechanisms for target recognition given that it interacts with several